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PREPARATION AND PROPERTIES OF BOVINE, MONKEY, AND RAT GROWTH HORMONES

[Following is the translation of an article by T. M. Shulkova and V. N. Orekhovich, Institute of Biological and Medical Science, AMN, USSR, Moscow, published in the Russian-language periodical Biokhimiya (Biochemistry), Vol 32, No 1, 1967. It was submitted on 27 Dec 1965.]

It is known that growth hormones, isolated from the pituitary of different species of animals, differ in their chemical composition and in their physico-chemical, immunological, and biological properties [1-5], and the hormones of Primates differ especially sharply from the somatotropins of other species of animals.

In the present article data are given which relate to methods of preparation of somatotropic hormones from a bull, rat, and green marmoset, and their comparative physico-chemical and biological characteristics are given.

Method

The growth hormone was obtained from the pituitary of a bull by the method of Wilhelmi [6], and then subjected to gel filtration in a column of Sephadex G-100 at 0-4°. 200 mg of the resulting crystalline preparation of bovine STG was diluted in 1 ml of 0.1 M acetic acid and placed on a column (3 x 75 cm) of Sephadex G-100 which was equilabrated with 0.1 M acetic acid. Fractions of 5 ml each were eluted from the column. Growth hormones from the pituitary of the green marmoset and rat were obtained in the following manner. [Translator's note. Probable definition of the Russian acronym STG is somatotropnyy gormon or somatotropic hormone.]

30 g of quick-frozen pituitary was homogenized in a glass homogenizer in a solution of $Ca(OH)_2$, pH 7.0-7.2 and the homogenate was extracted with 200 ml of this solution in the cold while being mixed for 20 hours. The extract was separated by centrifuging and the precipitate was rinsed with 100 ml of a $Ca(OH)_2$ solution. An equal amount of a saturated solution of $(NH_4)_2SO_4$ was added to the supernatant liquid. Precipitation with ammonium sulfate was carried out in the cold with mixing for an hour. The precipitate was collected by centrifugation, dissolved in 200 ml of distilled water, and dialyzed against distilled water for 46 hours. The pH of the solution was brought to 4.5 with the help of 1 n HCl and the resulting precipitate was centrifuged. The pH of the supernatant liquid was brought to 5.5 with the help of 1 n NaOH and the

precipitate was centrifuged. In the supernatant liquid the amount of protein was determined and the concentration of protein in the solution was brought to 0.2%. A 50% solution of ethanol up to an end concentration of alcohol of 5% was slowly added to the solution while being stirred. The precipitate was centrifuged and a 50% solution of ethanol up to an end concentration of 25% was added to the supernatant liquid during rapid stirring. The precipitate was collected by centrifugation, diluted in distilled water, pH 7.0, and lyophilized.

The resulting preparations were subjected to gel filtration in the cold on a column (2 x 50 cm) of Sephadex G-100, equilibrated with 0.1 M acetic acid. 80 mg of protein, diluted in 1 ml of 0.1 M acetic acid, was placed in the column and fractions of 5 ml were collected.

Electrophoresis was carried out in starch gel by the method of Smitis in a Poulik buffer system [7] (for the gel: 0.076 M of tris-buffer, 0.005 M citric acid; for electrode vessels: 0.3 M boric acid and 0.06 n NaOH) at 4° for 4 hours at a voltage of 22 V/cm.

Sedimentation coefficient of the somatotropins was determined in a Khitachi analytic ultracentrifuge. Experiments were conducted for 1.5 hours at a velocity of 59,780 rpm in 0.1 M borate buffer, pH 9.93; concentration of protein was 0.8%.

Diffusion coefficient was determined with the help of a Spinko A analytic ultracentrifuge in a cell for the artificial formation of a boundary at a velocity of 12,590 rpm for 2 hours in 0.1 M borate buffer, pH 9.93, with a concentration of protein of 0.8%. Calculation of the value of the diffusion coefficient was carried out by the method of area and maximum ordinate. *

* We express our thanks to A. D. Morozkin for carrying out the experiments on analytic ultracentrifugation.

N- and C-terminal amino acids of bovine, rat, and monkey growth hormones were determined by the dinitrofluorobenzene and carboxylpeptidase method [8].

Amino acid analysis of the preparations was conducted by the method of Spackman and associates [9] in a Khitachi amino acid analyzer.

Samples were hydrolyzed in 6 n HCl in vacuum-sealed ampoules for 24 hours at 110°. Tryptophan was determined spectrophotometrically [10]. Cystine was determined after oxidation of the hormone with performic acid [11].

The immunological reaction of somatotropins of bulls, monkeys, and rats with rabbit antiserum to human growth hormone * was carried out by the method of Ouchterlony in a thin layer of agar gel [13].

* Rabbit antiserum to human growth hormone was obtained by M. Lala Bel'kinyy at the Institute of Experimental Endocrinology, AMN USSR, Moscow.

The biological activity of the preparations was determined by the tibial test [13] and by a method, based on measurement of the intensity of incorporation of radioactive proline in collagen of the skin of hypophysectomized rats [14].

Female white rats weighing 70-80 g were hypophysectomized by the parapharyngeal method under ether anesthesia. After 12-14 days after the operation the hypophysectomized rats received daily for 4 days 10 micrograms of growth hormone. 24 hours after the last injection of STG the rats received intraperitoneally C^{14}-proline on the basis of 2.5 microcuries per 100 g of animal weight. After 24 hours the rats were sacrificed, the tibial bone was extracted, and it was split into sagittal plates and stained with 2% $AgNO_3$. Using a calibrated eyepiece, the width of the noncalcified section of epiphyseal cartilage was measured.

The results of testing were subjected to statistical treatment according to Styudent.

Results of the Investigation

By gel filtration of somatotropins through Sephadex G-100 the preparations were separated into two fractions, of which only one possessed hormone activity (figures 1 and 2). Out of 200 mg of bull STG 80 mg of protein was obtained which possessed biological activity; out of 30 mg of the ethanol fraction of rat and monkey STG 30 mg of active preparation were obtained from each.

During electrophoresis of the hormones in starch gel one component was revealed (Figure 3). This testified to the homogeneity of the preparations obtained. Somatotropic hormones from the hypophysis of rats and monkeys have a similar electrophoretic mobility.

During ultracentrifugation of the preparations one symmetrical peak was revealed with a sedimentation coefficient of 3.05S for bull STG, 2.35S for monkey STG, and 2.2S for rat STG.

In order to evaluate the molecular weights of the hormones experiments were set up for determining the diffusion coefficients.

Here the following data were obtained: diffusion coefficient for bull STG was equal to $7.0 \cdot 10^{-7}$, monkey STG - $6.9 \cdot 10^{-7}$, and for rat STG - $7.8 \cdot 10^{-7}$ cm^2/s.

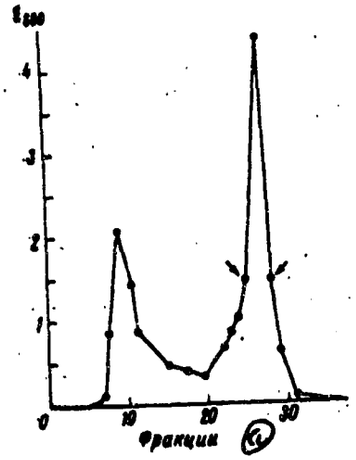

Fig. 1. Gel filtration of growth hormone from a bull through Sephadex G-100. The arrows show the active fraction taken for the investigation. (a) - fractions.

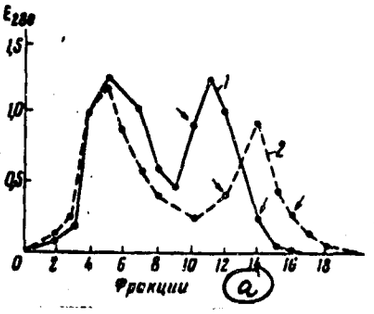

Fig. 2. Gel filtration of growth hormone from monkeys and rats through Sephadex G-100. 1 - STG of monkey; 2 - of rats. Arrows show the active fraction taken for investigation. (a) - fractions.

On the basis of data from sedimentation and diffusion, molecular weights were determined for the growth hormone of bulls (45,700), green marmoset (30,000), and the growth hormone of rats (27,000). As a result of possible errors when using the above method for determining the diffusion coefficient, the values of molecular weights for rat and marmoset STG should be viewed as preliminary. During determination of molecular weight we detected significant differences for monkey STG. According to the data of Li [15] the molecular weight of STG from Rhesus monkeys equals 25,400; according to our data the molecular weight of growth hormone for the marmoset equals 30,000.

Dinitrofluorobenzene and carboxylpeptidase methods were used for determining N- and C-terminal amino acids of the somatotropins. Growth hormones of rats and green marmosets contain phenylalanine as the N- and C-terminal amino acid. Bovine somatotropin has two N-terminal amino acids (phenylalanine and alanine) and one C-terminal amino acid (phenylalanine).

Thus the growth hormones of rats and green marmosets are similar

based on their physico-chemical properties and are different from bovine growth hormone. STG from rats and monkeys have a lesser molecular weight, a more acid isoelectric point [15, 16], and only one N-terminal radical (phenylalanine).

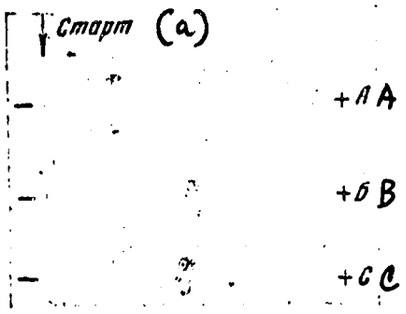

Fig. 5. Electrophoresis of growth hormones of bulls, monkeys, and rats in starch gel. A - bovine STG; B - monkey and C - rat. Key: (a) Start.

Table 1

Amino acid composition of growth hormones of bulls, monkeys, and rats

Аминокислоты (a)	Бык (b)			Обезьяна (c)			Крысы (d)		
	г/100 г белка (e)	Количество остатков/моль (f)		г/100 г белка (e)	Количество остатков/моль (f)		г/100 г белка (e)	Количество остатков/моль (f)	
g Лизин	7,16	25	23*	5,65	12	12*	6,06	12	11**
h Гистидин	2,06	8	7	1,9	4	5	1,08	3	3
i Аргинин	8,00	24	26	6,9	13	13	6,74	10	9
j Аспарагиновая кислота	10,67	40	35	10,15	25	26	8,88	18	17
k Треонин	4,09	22	26	4,21	12	13	3,76	8	8
l Серин	4,83	23	22	6,1	19	20	5,04	12	12
m Глютаминовая кислота	13,23	47	50	13,36	30	33	11,07	20	24
n Пролин	3,51		14	4,92	14	10	4,6	11	9
o Глицин	3,3	20	20	4,71	19	15	3,79	12	11
p Аланин	5,34	30	31	3,8	14	11	3,91	11	14
q Цистин	1,02	4	4	2,8	4	4	1,72	2	2
r Валин	2,8	12	14	5,74	16	9	5,61	13	9
s Метионин	2,08	7	7	1,87	4	6	1,79	4	3
t Изолейцин	2,82	11	76	3,93	10	41	3,67	8	7
u Лейцин	15,98	61		10,7	27		11	22	18
v Тирозин	4,34	12	11	5,75	10	7	3,62	6	5
w Фенилаланин	8,81	27	27	6,0	12	16	5,83	9	9
x Триптофан	1,22	3	3	1,23	2	1	1,2	1	—
	103,05	389	396	100,02	247	241	91,87	182	171

Key: (a) Amino acids; (b) Bull; (c) Monkey; (d) Rat; (e) g/100 g protein; (f) Quantity of radicals/mole; (g) Lysine; (h) Histidine; (i) Arginine; (j) Asparaginic acid; (k) Threonine; (l) Serine; (m) Glutamic acid; (n) Proline; (o) Glycine; (p) Alanine; (q) Cystine; (r) Valine; (s) Methionine; (t) Isoleucine; (u) Leucine; (v) Tyrosine; (w) Phenylalanine; (x) Tryptophan.
* Data from Li [15]. ** Datat from Reisfeld [16].

Table 1 gives the results of an amino acid analysis of the preparations obtained by us, and also data from Li for bovine and Rhesus STG [15] and data from Reisfeld for rat STG [16]. It can be seen from Table 1 that rat and marmoset STG have a similar amino acid composition, which differs significantly from the amino acid composition of bovine growth hormone. A predominance of dicarboxylic amino acids and leucine is characteristic for the amino acid composition of all the preparations. Certain deviations between our data and the results obtained by other investigators may be explained most likely by the diverse degree of purity of the preparations analyzed.

During an immunological investigation of the isolated growth hormones it was demonstrated that the antiserum to human STG interacted only with marmoset somatotropin. Here the line of precipitation, formed by the antiserum and green marmoset STG was somewhat weaker than the line of precipitation, formed by antiserum and human STG. Consequently the somatotropin of the green marmoset has a similar, but not identical, antigenic structure with the somatotropin of man.

Table 2

Activity of growth hormones of bulls, monkeys, and rats based on the tibial test and on the incorporation of C^{14}-proline in the skin collagen of hypophysectomized rats.

(a) В каждом опыте использовано по 6 животных

(b) Гормон, 40 мкг (общая доза)	(c) Ширина тибиального хряща, мк		(d) Включение пролина в коллаген, имп/мин/5 мг белка
(e) Контроль	150±5		78± 2
(f) Бык	224±11	258±8*	152±12
(g) Обезьяна	231± 9	272±8*	159±13
(H) Крыса	227± 9	258±10*	157± 8

Key: (a) 6 animals were used in each test; (b) Hormone, 40 micrograms (total dose); (c) Width of tibial cartilage, microns; (d) Incorporation of proline in collagen, imp/min/5 mg of protein; (e) Control; (f) Bull; (g) Monkey; (H) Rat.
* Data were obtained after administration of 100 micrograms (total dose) of hormones.

As can be seen from Table 2, the resulting preparations possess a high degree of biological activity. Daily administration of 10 micrograms of a preparation of bovine, monkey, and rat STG to hypo-

hypophysectomized rats caused a considerable increase in the width of epiphysial cartilage of the tibial bone and increased by 100% the intensity of incorporation of labeled proline in the skin collagen of hypophysectomized rats.

Conclusions

Growth hormones were obtained from bovine, green marmoset, and rat pituitaries by various methods. They were homogeneous based on data from ultracentrifugation and electrophoresis. The somatotropins from the green marmoset and rats have a similar amino acid composition; phenylalanine is the N- and C-terminal amino acid of these hormones. The molecular weight of rat and green marmoset STG equals respectively 27,000 and 30,000.

In spite of significant differences in the physico-chemical properties, preparations of bovine, monkey, and rat growth hormones exert a similar biological action on hypophysectomized rats. ( )

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